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Artigo original

Evaluation of *Listeria monocytogenes* in frescal cheese traded in the northwestern region of the state of Paraná

Avaliação de *Listeria monocytogenes* em queijo frescal comercializado na região noroeste do estado de Paraná

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A B S T R A C T

Listeria monocytogenes is responsible for listeriosis, a food zoonosis that causes episodes of food poisoning all around the world; lives in the soil, vegetation, silage, nasal secretion and also in the intestinal flora of many animals. The transmission occurs through material contaminated with feces, urine, aborted fetuses, uterine discharge, milk and its derivatives, mainly fresh cheese, since they are made with unpasteurized milk, are not cured, have low percentage of salt and high humidity, factors favorable to rapid microbial growth and consequent food poisoning to consumers. Frescal cheese is widely consumed due to its low cost, palatability and in natura state, attending to the growing demand for foods with minimal processing. This article aimed to evaluate the presence of *Listeria monocytogenes* in samples of Frescal cheese marketed in the northwest region of the state of Paraná. Twenty - one samples of Frescal cheese marketed in different establishments were purchased. The samples were stored in thermal boxes, duly identified and sent to the Laboratory of Control and Inspection of Quality and Food (LACOMA) at the Federal University of Paraná (UFPR), Palotina campus, where the analyzes were carried out. All 21 analyzed samples did not present *Listeria monocytogenes*, according to the microbiological standard for high humidity cheese (55%), as defined by the National Agency of Sanitary Surveillance (ANVISA).

R E S U M O

Listeria monocytogenes é responsável pela listeriose, uma zoonose alimentar que causa episódios de intoxicação alimentar em todo o mundo. Vive no solo, vegetação, silagem, secreção nasal e também na flora intestinal de muitos animais. A transmissão ocorre por meio de material contaminado com fezes, urina, fetos abortados, descarga uterina, leite e seus derivados, principalmente o queijo fresco, uma vez que são fabricados com leite não pasteurizado, que não são curados, além de apresentarem baixa porcentagem de sal e alta umidade, fatores favoráveis ao rápido crescimento microbiano e consequente intoxicação alimentar aos consumidores. O queijo frescal é amplamente consumido devido ao seu baixo custo, palatabilidade e estado *in natura*, atendendo à crescente demanda por alimentos com mínimo processamento. Este artigo teve como objetivo avaliar a presença de *Listeria monocytogenes* em amostras de queijo frescal comercializado na região noroeste do estado do Paraná. Foram compradas 21 amostras de queijo frescal comercializado em diferentes estabelecimentos. As amostras foram armazenadas em caixas térmicas, devidamente identificadas e encaminhadas para o Laboratório de Controle e Inspeção de Qualidade e Alimentos (LACOMA) na Universidade Federal do Paraná (UFPR), campus de Palotina, onde as análises foram realizadas. Todas as 21 amostras analisadas não apresentaram presença de *Listeria monocytogenes*, em conformidade com o padrão microbiológico para queijo de alta umidade (55%), conforme definido pela Agência Nacional de Vigilância Sanitária (ANVISA).

K E Y W O R D S
Zoonosis; Public Health; Microbiological Contamination.

P A L A V R A S - C H A V E
Zoonose; Saúde Pública; Contaminação Microbiológica.

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INTRODUCTION

According to the World Health Organization (WHO), listeriosis is a food-borne disease, usually infectious or toxic, caused by agents that come in contact with the human organism through the ingestion of contaminated food or water, being one of the main causes of death in developing countries, killing approximately 1.8 million people, mainly children, per year (VEIGA et al., 2009). It is estimated that approximately 30% of the population in developed countries is affected by this type of disease (NEWELL et al., 2010).

Cheese is considered a frequent vehicle for food-borne pathogens, with handmade fresh cheese being especially common, since most of them are made from raw milk and do not go through maturation process. The microbial contamination of these products is highly relevant for the industry, due to economic losses, and to public health, due to the risk of causing food-borne diseases (FEITOSA et al., 2003).

Handmade fresh cheese, as well as presenting high humidity and being highly perishable, it is not cured, presenting low salt content and intense handling, providing very suitable conditions for bacterial contamination, survival and multiplication. Such contaminating bacteria include the highly pathogenic *Listeria monocytogenes*, which is able to produce microbial metabolites and cause intoxication and/or food poisoning in humans (CÂMARA et al., 2002).

The disorders caused by *L. monocytogenes* include sepsis, meningitis, encephalitis, cervical or intra-uterine infection in pregnant women, abortion or premature birth. Other damages may occur, such as endocarditis, granulomatous lesions on liver and other organs, internal or external abscesses. Gastrointestinal symptoms such as nauseas, vomit and diarrhea may precede or accompany the most severe manifestations of the disease. Mortality rate in newborns is around 30%; adults present a 50% mortality rate when presenting sepsis, reaching up to 70% in the case of meningitis (BURALL et al., 2005).

Due to the high mortality rates in severe cases, *L. monocytogenes* is an agent that raises the attention of governmental agencies responsible for the sanitary control, as well as of the scientific community in the food area. Listeriosis outbreaks and cases have been associated to several food items, both from vegetable and also from animal source. Among the outbreaks caused by dairy products, fresh cheese is considered as posing the greatest risk, and has already been involved in several outbreaks (BORGES et al., 2009).

The purpose of this study was to evaluation, isolate and identify the presence of *Listeria monocytogenes* in 21 samples of frescal cheese purchased in the northwestern region of Paraná, assessing the contamination and food poisoning risk by their consumption for the population of that region, since little information is available regarding the characteristics of this micro-organism in food-borne diseases (FBD), mainly in fresh cheese that is broadly produced and consumed in that area.

Northwestern Paraná has 26,400 km², a humid subtropical weather and soil with low and medium clay content. This region has 69 municipalities and 630,421 inhabitants, and it is border to the states of São Paulo and Mato Grosso do Sul. The northwestern region of Paraná measures 24,488.68 km^2 (12% of the area of the state) and has a population density of 25.7 $inhab/km^2$. The northwestern region holds approximately 6% of the population of the state and has a large industrial and agribusiness importance (IBGE, 2016). The state of Paraná is the third largest producer of milk in Brazil, accounting for 3.9 billion liters per year, representing the most important productive chain for family farmers, mainly those in the northwestern region of the state, where dairy products, especially frescal cheese, are widely produced and are a traditional food for most of the population, with significant growth in both trade and consumption in recent years (BRASIL, 2016).

MATERIAL AND METHODS

The *L. monocytogenes* survey was performed according to the Normative Instruction No. 62 (BRASIL, 2003), by means of selective enrichment (primary and secondary), plating and isolation. Biochemical assays were performed in order to confirm the bacteria.

A total of 21 samples of fresh cheese from commercial fairs in the northwestern region of Paraná, handcrafted and from different producers, were purchased between August 2015 and May 2016. The samples were stored in thermal boxes, properly identified and forwarded to the Laboratory of Control and Inspection of Quality and Food (LACOMA) at Universidade Federal do Paraná (UFPR), Palotina campus, for further analyzes.

The samples were first submitted to selective enrichment, 25 gram from each sample were homogenized in 225 mL enrichment broth for *Listeria* (LEB-Merck) and incubated for 24 hours in at 30º C. Then, a second selective enrichment was used, using 0.1 mL of the homogenized solution, inoculated in a tube containing 10 mL of Fraser broth (LEB-Merck), incubated at 35°C for 24 hours. After incubation in the Fraser broth, selective plating was performed for isolation, using Oxford Agar (LEB-Merck) and left at 35°C for 24-48 hours. After this process, three to five characteristic colonies, black colonies with dark halo, were selected for purification. These colonies were streaked onto Tripticase Soy Agar plates with 0.6% yeast extract (TSA-YE) (LEB-Merck) and incubated in furnace at 35°C for 24-48 hours. For the biochemical identification, one characteristic colony (blue color and shattered glass aspect) from each TSA-YE plate was selected and inoculated in tubes containing TSA-YE. From the tubes, catalase, motility

(in furnace at 25 ºC for 7 days), sugar fermentation: Rhamnose, Mannitol and Xylose (in furnace at 30 ºC for 36 hours) and Hemolysis (in furnace at 35 °C for 24 - 48 hours) biochemical assays were performed.

RESULTS AND DISCUSSION

All of the 21 samples analyzed presented absence of *Listeria monocytogenes* in 25-gram samples, being compliant to the microbiological standard for high humidity cheese (55%), as established by the Brazilian National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária - ANVISA), through RDC No. 12 (2001). This pathogen has been observed in this type of product (BRASIL, 2001), with the products presenting satisfactory hygiene and sanitary conditions and showing that the products have good handling and trading practices, and do not pose contamination or infection risks to the consumers of fresh cheese in the region. However, preventive measures and good handling practices for food must always be enforced in order to maintain this microbiological standard and not expose consumers to risk.

The low population of *L. monocytogenes* and the sub lethal injury caused by the processing are factors that hinder its recovery in the enrichment broth. The presence of coliform may also influence the *L. monocytogenes* population in frescal cheese, hindering its proliferation, and even causing difficulty in its detection. Thus, a negative result does not guarantee the absence of the pathogen (ARAGON-ALEGRO et al., 2006).

Similar results were obtained by Peresi et al. (2001) when assessing 30 frescal cheese samples purchased in street markets in the city of São José do Rio Preto, in the state of São Paulo, all samples presented absence of *L. monocytogenes*, Alves (2013) also not detected the presence of *L. monocytogenes* in 60 samples analyzed from street markets in the city of Volta Redonda, RJ.

Salotti et al. (2006) assessed 30 artisan cheese samples purchased in the city of Jaboticabal, in the state of São Paulo; while Brant, Fonseca and Silva (2007) analyzed 40 samples of cheese produced in the region of Serro, in the state of Minas Gerais, and neither study detected the presence of *L. monocytogenes*. Such data corroborate our study, where the presence of *L. monocytogenes* was not detected in the 21 samples analyzed. The study by Pinto et al. (2011) also resulted in the total absence in the 40 samples of frescal cheese analyzed in the city of Santa Helena, in the state of Paraná.

The results of this paper do not corroborate with those from Silva; Vilardi; Tibana (1998), that detected a high incidence in 7 (41.2%) of the 17 samples assessed for *L. monocytogenes* in artisan frescal cheese produced in Rio de Janeiro, or with the study developed by Silva et al. (2012), that analyzed 15 samples of frescal cheese from a supermarket located in the city of Barra Mansa, in the southern region of Rio de Janeiro, where 5 (33.3%) samples presented *L. monocytogenes*, from a total of 15 samples analyzed.

The results of this paper also differ from those found by Carvalho; Viotto; Kuaye (2005), who, upon analyzing 93 frescal cheese samples undergoing different processing methods, traded in the city of Campinas, in the state of São Paulo, reported the presence of *Listeria* spp. in 11 (11.8%) samples, and the isolate was identified as *L. monocytogenes* in three (3.2%) of them.

Studies on the incidence of *L. monocytogenes* in Brazil report different contamination indexes and a broad variance, even in surveys performed within the same state. Abrahão (2008) analyzed 90 samples from different types of cheese traded in the state of Paraná using the visual immunoprecipitation assay (VIP) and found 3.3% (3/90) samples positive for *L. monocytogenes.* Bernardi (2014), analyzing 77 frescal cheese, also originated from cheese producing cities in the state of Paraná, found 9 (11.7%) contaminated samples. Such data is divergent from this paper in the northwestern region, where the samples were negative for the presence of *L. monocytogenes*.

In Brazil, human listeriosis is under diagnosed and under notified, and therefore, there are no official statistics for listeriosis cases. However, several papers show the circulation of *L. monocytogenes* in humans, causing infection and severe public health issues (BARANCELLI et al., 2011).

Hofer, Reis; Hofer (2006) performed a phenotypic analysis on *L. monocytogenes* strains in several regions of the country, with the South and Southwest presenting the highest number of isolates for *L. monocytogenes* (87.8%), suggesting that this fact may be associated to different eating habits, such as the high consumption of *in-natura* products or the high production and trade of dairy and non-industrialized animal-origin products in street markets.

Schwab and Edelweiss (2003), in a retrospective study in the pathology sector of a teaching hospital in the city of Porto Alegre, analyzed 148 human placenta samples from miscarriages and premature births using the immuno-histochemical technique (IHQ). In that study, 50 placentas (33.7%) were positive for the presence of *L. monocytogenes*, from which 66.6% were resulting from miscarriages and 33.3% from premature births.

The infecting dose for *L. monocytogenes* has not been determined, but it is believed to vary according to the strain, the host's susceptibility and the food matrix involved. Bortolussi (2008) suggests that the infecting dose of this food pathogen is estimated between 10-100 million colony forming units (CFU) in healthy hosts, while only 0.1-10 million CFU is necessary in immunodeficient individuals. However, Barancelli (2011) argues that the dose required to cause diseases in susceptible individuals can be of approximately 100 to 1000 pathogen cells, and even lower for immunodeficient individuals.

Listeriosis is most frequently bacteria observed in developed countries, since it is influenced by the change in the population's lifestyle, who increase the consumption of "ready for consumption" processed foods, usually stored under refrigeration temperatures (SWAMINATHAN and GERNER-SMIDT, 2007).

Listeriosis presents a considerable mortality rate, ranging between 20% - 30%, with 90% hospitalization rates. This mortality rate can be even higher in risk groups, such as immunodeficient, elderly and patients with central nerve system infections, reaching up to 70%, justifying its importance among the food-borne diseases (LITTLE et al., 2012).

Most diagnosed cases are concentrated in Europe and the United States. The number of reported cases has been increasing, probably due to a better laboratory diagnosis and an increase in the susceptible population, together with the high prevalence of the bacteria in the environment and handling habits, inappropriate preparation and storage of food (OSAILI et al., 2012).

According to Lenhart et al. (2008), the chance of a pregnant women becoming infected by *L. monocytogenes* is fourteen-fold greater than for a non-pregnant woman belonging to a healthy population. This aspect is associated to the reduction of the immunity mechanism required to keep the pregnancy.

Listeriosis has a long incubation period, which can reach up to 90 days, hindering the identification of the pathogen and the tracking of the contaminated food that caused the disease (GANDHI and CHIKINDAS, 2007).

In the food industries in Brazil, there has been an increasing interest in the detection of *L. monocytogenes* due to scientific papers reporting the association of the bacteria present in the processing environment with the final product and the risk of contamination and infection to the consumer. Studies based on DNA have shown that certain *L. monocytogenes* strains are established in food industries, where they remain for months or even years, constituting permanent sources of contamination. The persistence of strains in the industrial environment is related to the adaptability of the bacteria and the formation of biofilms (BARANCELLI et al., 2011), emphasizing that the contamination by *L. monocytogenes* may also happen in industrial products, not only in artisan ones.

In general, there is a low occurrence of *L. monocytogenes* in cheese in Brazil, but literature shows that several authors have found high coliform populations in the analyzed products and suggest that the low occurrence of *L. monocytogenes* may have resulted from the high coliform populations in these products, since they compete with *Listeria* in the food and interfere in the isolation of the pathogen (BARANCELLI et al., 2011).

Until now, there are few scientific data and literature available in Brazil to prove or correlate the consumption of contaminated food with cases of listeriosis. One of the factors that possibly contribute to this situation is that many public health laboratories do not routinely perform the survey of this pathogen in clinical or food samples, and many physicians are not aware of the importance of the bacterium. Additionally, the disease's long incubation period hinders the elucidation of food-borne outbreaks (BERNARDI, 2014).

CONCLUSION

All samples analyzed were absent for *Listeria monocytogenes* in 25 gram samples, in compliance with the microbiological standard for high-humidity cheese, as defined by the Brazilian National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária (ANVISA).

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